THE BIOLOGICAL DEGRADATION OF SOLUBLE CELLULOSE DERIVATIVES AND ITS RELATIONSHIP TO THE MECHANISM OF CELLULOSE HYDROLYSIS

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The use of soluble cellulose derivatives in the form of gels for pharmaceutical and other purposes depends in part upon the resistance of the preparation to liquefaction. Reports have reached us that products containing soluble carboxymethyl cellulose (CMC) liquefied on standing. From one of the deteriorated samples Woodward (1948) isolated *Aspergillus niger* and *Aspergillus flavus*; from another preparation Freeman *et al.* (1948) obtained gram-negative bacteria, which on reinoculation again liquefied the CMC. Contaminated hydroxyethyl cellulose preparations behave in a similar fashion. From one sample Harry (1948) isolated two bacterial organisms. In a study of the availability of cellulose derivatives to *Myrothecium verrucaria*, Siu *et al.* (1949) found that CMC-Na salt and methocel (soluble methyl cellulose) were metabolized.

From our viewpoint several points appeared to require further elucidation. How widespread is the ability of microorganisms to utilize soluble cellulose derivatives as carbon sources? How do the number, nature, and location of the substituents affect the availability of the modified cellulose? And, finally, is there any relationship between the enzymatic hydrolysis of cellulose derivatives and the hydrolysis of cellulose itself? These are the questions to which the following data are partial answers.

METHODS

Carboxymethyl cellulose, sodium salt (CMC), hydroxyethyl cellulose (HEC), and methyl cellulose have been employed as substrata. These are water-soluble cellulose ethers with very low reducing values (1 per cent solutions give a reducing value as glucose of less than 0.01 mg per ml). As in ethers, it is difficult to split off the substituted side groups by acid hydrolysis. Indeed, the anhydroglucose units of the cellulose chain are hydrolyzed before removal of the side groups. Most of our efforts have been devoted to work with CMC because of the ready availability of samples[1] of known degrees of substitution (DS) and of known degrees of polymerization (DP). By degree of substitution is meant the number of substituted groups per anhydroglucose unit. The degree of polymerization refers to the number of anhydroglucose units per molecule.

In addition to the substrata already mentioned, ground cotton duck and bleached cotton sheeting were used in many cases. Of the two, the former appears to be superior as a carbon source, perhaps because of the growth factors

[1] We wish to thank the Hercules Powder Company for its co-operation in supplying the various CMC samples.

it contains. When filtrates were being assayed for their hydrolytic action on cellulose, ground dewaxed cotton sliver was chosen, since it represents a readily available source of comparatively unaltered native cellulose. The grinding was done in a Wiley mill, and the product is somewhat less susceptible to enzymatic attack than ball-milled cotton. The procedures followed to obtain active filtrates are summarized briefly: (1) To the mineral salts yeast extract medium[2] is added ground gray duck or soluble cellulose derivative at a concentration of 0.5 to 1.0 per cent. The salts medium is heated and added to the cellulose derivatives in a Waring blender to effect solution. One hundred ml of the mixture is placed in 500-ml Erlenmeyer flasks and autoclaved for 20 minutes. (2) One ml of a water suspension of spores is added to each flask. After inoculation the flasks are incubated on a shaker for 7 to 10 days at 30 C (40 C for *Aspergillus fumigatus*). (3) At the end of the incubation period the solutions are filtered through fritted glass filters of medium porosity. The filtrates are always clear. One ml

TABLE 1

Soluble cellulose derivatives

NAME	TYPE	SOURCE	DS	MEAN DP
Carboxymethyl cellulose, Na salt (CMC-Na)	4 WH	du Pont	0.7	—
	50 T	Hercules	0.52	150
	70 H	Hercules	0.82	200
	70 M	Hercules	0.89	150
	70 L	Hercules	0.88	125
	90 M	Hercules	0.99	150
	120	Hercules	1.2	150
Methyl cellulose (methocel)	M15370	Dow Chemical	1.77	
Hydroxyethyl cellulose (cellosize)	WPLM	Carbide & Carbon	2.25	

of 1 per cent merthiolate[3] is added per 100 ml of filtrate, and the solutions are stored at refrigerator temperature until required.

The index of activity of the culture filtrates has been the amount of reducing sugars (as glucose) produced from CMC 50 T under a specified set of conditions. We are restricting the use of the term Cx to the enzyme capable of hydrolyzing the 1,4-β-glucosidic linkage as found in cellulose and as measured by the amount of reducing sugars obtained by hydrolysis of CMC. The term "cellulolytic" is used in its broader sense indicating the ability to attack or hydrolyze native cellulose (cotton and filter paper). Cx alone is unable to hydrolyze these materials. The method of determination of the Cx activities of filtrates as performed in this laboratory follows: (1) To 100 ml of 1 per cent CMC 50 T, add 20 ml M/2 sodium hydroxide citric acid buffer (pH 5.0) and 60 ml water. Mix well and place 9 ml of the mixture and 1 ml of culture filtrate into test tubes,

[2] Mineral salts yeast extract medium: NH_4NO_3, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; 10 ml M/1 KH_2PO_4-K_2HPO_4 at pH 5.8; yeast extract, Difco 0.1 g; distilled water to 1,000 ml.

[3] Merthiolate (Lilly) = sodium ethylmercurithiosalicylate.

using duplicate test tubes for each filtrate under examination. (2) Mix well. Immediately remove 1 ml for sugar determination (zero incubation time). (3) Incubate mixture in water bath at 50 C for 2 hours. After incubation remove 1 ml for sugar determination. (4) Activity is recorded as an increase in reducing sugar (as glucose) during incubation.

Sugars are determined by the colorimetric, dinitrosalicylic acid (DNS) method of Sumner and Somers (1944), which covers the range 0.05 to 0.90 mg per ml. In the course of this work it was found that heavy metals interfere with this method. Copper concentrations as low as 4×10^{-5} M gave an apparent increase in glucose; silver and mercury at 0.001 M gave values that were low. The effect of copper, however, is not apparent when the reducing value is zero, as normally found in the controls. For some time we felt that copper actually had a stimulatory effect on the enzyme Cx, but we discovered the artifact at a later date.

RESULTS

Growth of various microorganisms on soluble cellulose derivatives. Each cellulose derivative was dissolved in a mineral salts solution containing 0.01 per cent of yeast extract. After autoclaving, the pH of the mixture was about 6.3. In these tests the concentration of the derivative was such as to result in a solid (or highly viscous) medium. The ability to utilize the substrate was estimated by the amount of growth and by liquefaction of the cellulose derivative.

Growth of most organisms was very good on CMC preparations of low degrees of substitution with liquefaction proceeding rapidly from the top of the medium to the bottom. When the DS was above 1.0, however, growth was slight, and no liquefaction occurred within 30 days.

Two per cent solutions of methocel in the usual salts yeast extract medium are liquid at room temperature but solid at 60 C. At the end of the period of incubation with the organisms, the tubes were shaken and placed in a bath at 60 C for 15 minutes. The medium solidified in the controls and in those in which no action had occurred. Where hydrolysis had taken place, complete solidification failed to occur, even when the temperature was raised to 80 C. The amount of growth occurring in the methocel medium was slight, comparable to that on CMC of high DS. Actual utilization of methocel in these cases is questionable, although it appears that slight hydrolysis of the polymer has been effected by a few of the microorganisms.

A 10 per cent solution of hydroxyethyl cellulose made a very viscous but not solid medium. "Cellosize" WPLM was an excellent substratum for most microorganisms. This odd behavior, which is apparently inconsistent with the theory that a DS greater than one imparts resistance, will be discussed later.

Thirty organisms were tested on the above substrata. Cellulolytic fungi and bacteria and noncellulolytic fungi behaved in essentially the same manner. The four noncellulolytic bacteria tested, however, were unable to utilize any of the derivatives.

In the next series of experiments the organisms were grown in shake flasks on carboxymethyl cellulose of low DS. The culture fluid was filtered through

sintered glass, and the cell-free filtrates were tested for their ability to hydrolyze CMC. The possession by the filtrates of an enzyme system capable of hydrolyzing the 1,4-β-glucosidic linkage of cellulose and its derivatives is clearly indicated (table 2). A drop in viscosity of the solution takes place first and is fol-

TABLE 2

Production of the hydrolyzing enzyme, Cx, by various microorganisms

ORGANISM	REDUCING SUGAR FROM CMC (AS GLUCOSE) MG/ML*	
	Expt. I	Expt. II
Noncellulolytic		
Aspergillus flavus, QM-10e	0.40	0.18
Aspergillus niger, QM-458	0.19	0.00
Aspergillus sydowi, QM-31c	0.36	0.33
Cellulolytic		
Actinomyces sp., QM-B814	—	0.20
Aspergillus fumigatus, QM-45h	0.45	0.29
Aspergillus luchuensis, QM-873	0.37	0.30
Cellulomonas sp., QM-B525	—	0.06
Myceliophthora lutea, QM-514	—	0.41
Myrothecium verrucaria, QM-460	0.54	0.39
Sporocytophaga myxococcoides, QM-B482	0.15	—

Experiment I: CMC 50 M used as substrate for growth and for enzyme activity.

Experiment II: CMC 70 M used as substrate for growth; CMC 14 WL as substrate for enzyme activity.

* Time of incubation of enzyme with substrate, 2 hours at 50 C.

TABLE 3

Relative rates of hydrolysis of soluble cellulose derivatives by various filtrates

SOURCE OF FILTRATE	SUBSTRATE FOR GROWTH	REDUCING VALUE AS GLUCOSE MG/ML*			
		CMC DS 0.52	CMC DS 1.2	HEC DS 2.25	Methocel DS 2.0
Syncephalastrum racemosum (contaminated)	CMC	0.68	0.00	0.53	0.04
Myrothecium verrucaria	CMC	0.51	0.03	0.40	0.03
Aspergillus flavus	CMC	0.28†	—	0.23†	0.06
Actinomyces sp	CMC	0.17†	—	0.14†	0.05
Myrothecium verrucaria	Cotton duck	0.83	0.03	—	0.01
Actinomyces sp	Cotton duck	0.66	0.00	—	0.00

* Four hours' incubation at 50 C; sugars by DNS method.

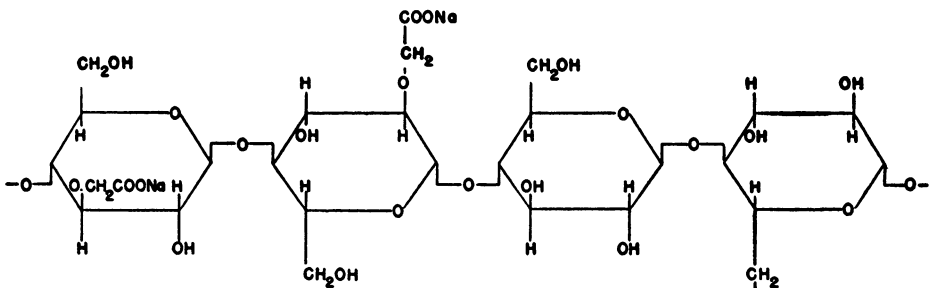
† These four samples received only 1 hour of incubation.

lowed by an increase in reducing compounds. As shown above, there is no qualitative correlation between the ability of an organism to attack native cellulose and its ability to produce the enzyme hydrolyzing CMC. The ability to produce Cx is widespread among microorganisms, the rate at which it is

formed being roughly the same for noncellulolytic as for cellulolytic organisms. To the best of our present knowledge none of the *Mucorales* are cellulolytic, and most of the isolates of this group examined by us are also incapable of hydrolyzing CMC. The noncellulolytic bacteria tested resemble the members of the *Mucorales* in being unable to grow on CMC. On the other hand, the noncellulolytic, as well as the cellulolytic, members of the genus *Aspergillus* are highly active on CMC and on some of the other cellulose derivatives.

Cx, produced by cellulolytic and by noncellulolytic organisms grown on CMC, is capable of hydrolyzing the hydroxyethyl cellulose used above, but it is unable to hydrolyze methyl cellulose of a similar DS (table 3). The presence of a similar enzyme capable of hydrolyzing hydroxyethyl cellulose was reported some years ago by Ziese (1931). His enzymatic preparations, from snail gut and from malt, also reduced the viscosity of the modified cellulose, but, unlike our filtrates, they did not produce reducing sugars.

Effect of DS and DP on enzymatic hydrolysis of soluble cellulose derivatives. In this portion of the work CMC-Na was used almost exclusively. The preparations varied in DP from 200 anhydroglucose units to less than 100, and in DS from 1.2 to 0.52. The viscosity of solutions of CMC is primarily a function of the DP, whereas the solubility is mainly dependent upon the DS. The formula of a sample of DS 0.75 may be represented as follows:



CMC, Na salt. Idealized portion of chain of DS 0.75, showing location of substituent at each of the three possible locations.

CMC is water-soluble at a degree of substitution as low as 0.4 to 0.5. From the data of Spurlin (1939), a uniform sample of DS 0.5 (CMC 50 T) would have about 40 per cent of the anhydroglucose units monosubstituted, 5 per cent disubstituted, and 55 per cent unsubstituted. On the other hand, a sample of DS 1.2 (CMC 120) would have only 10 per cent of the anhydroglucose units unsubstituted.

CMC is not readily hydrolyzed by dilute acid. A sample autoclaved for 15 minutes at 121 C with N/2 H_2SO_4 showed no increase in reducing value. Other samples kept at 50 C for 3 hours at pH values from 2.1 to 6.7 were likewise unaffected. Enzymatic hydrolysis, on the other hand, is rapid, whether it is measured as change in viscosity or as increase in reducing values.

In our experiments the degree of polymerization did not affect the rate of

enzymatic hydrolysis of the CMC preparations used. However, the range (DP 125, 150, 200) covered was narrow, and only three samples were used.

The degree of substitution has a decided effect on enzymatic hydrolysis as measured by the production of reducing compounds. Four samples of CMC of about the same DP (150) but varying in DS were available. Three of these were of known degrees of substitution, but the fourth (Hercules CMC 120) is a class designation, which means that the DS is about 1.2. When samples of these materials were hydrolyzed by filtrates of *Aspergillus fumigatus* for 2 hours at 50 C, the amount of reducing sugar obtained was an inverse function of the DS. The data (figure 1) indicate that the presence of one substituent on every anhydroglucose molecule renders the chain refractory to attack. Conversely, the lower the DS the greater the amount of reducing sugar obtained. As a substratum for enzyme studies, the CMC having the lowest DS consistent with solubility and viscosity is most desirable. In this set of samples, that having a DS of 0.52 is the most suitable. Since the solubility falls off rapidly below this level,

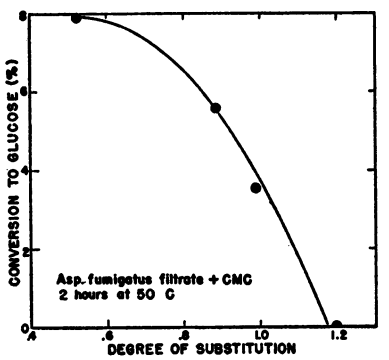


Figure 1. Action of culture filtrates on CMC of different degrees of substitution.

it is suggested that this material be chosen as the standard substratum for determining the Cx activity of cellulolytic filtrates. It has been estimated that the average degree of polymerization is about 150. A solution of 1 per cent concentration flows readily.

The data (table 3) indicate that what is true for *Aspergillus fumigatus* filtrates is true also for the filtrates of other organisms, namely, that the resistance of CMC to enzymatic hydrolysis is a function of the DS. Methyl cellulose has the resistance anticipated for compounds of DS over one. On the other hand, the relative ease with which organisms hydrolyze the highly substituted hydroxyethyl cellulose has caused us some concern. If it is assumed that the relationship between DS and hydrolysis found for CMC be applicable to HEC, the latter should have a DS of about 0.8 to 0.9 based on the amount of reducing sugar produced. Actually the first data supplied by the manufacturer indicated a DS of 2.25. Recently this figure has been changed to 1.47. The latter value was obtained by the method of Morgan (1946), which depends upon the liberation and estimation of ethyl iodide and ethylene. Morgan himself says, "Obviously the

method does not determine whether the hydroxyethyl ether groups are present as individual units or are built up of polyethylene oxide units of varying sizes. It seems more than probable that the latter is increasingly the case as the substitution is increased." We are convinced that the true DS of the hydroxyethyl cellulose sample tested by us is below 1.0 and that the polyethylene oxide side chains therefore average 2 to 3 units in length. That being so, the biological method as here used would appear to give a closer estimation of DS for hydroxyethyl cellulose than does the present chemical method. However, final proof of the DS may be furnished by some future chemical procedure.

The results confirm the data of Siu *et al.* (1949), which show that the degree of microbial resistance is directly related to the DS and is independent of the degree of polymerization. Recent data of Greathouse (1949), showing that the action of *Myrothecium verrucaria* on hydrocelluloses is independent of

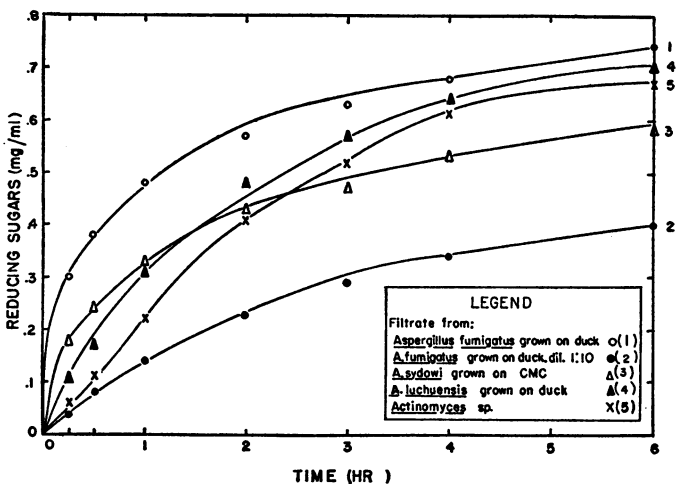


Figure 2. Effect of time on Cx activity. Filtrates incubated with CMC 50 T at 50 C, pH 5.1.

the DP over a large range (DP 120 to 1,675), are also in agreement with the experiments reported here.

Effect of time of incubation. A 2-hour incubation time has been selected in order that positive results may be obtained with filtrates of low activity. In actual practice many experiments have been performed using a 1-hour incubation and, in some cases, a 30-minute period. Under the conditions set forth above the minimum time may be estimated as that time required by the most active preparation in the test to produce about 0.5 to 0.6 mg per ml reducing sugar (as glucose). From the shape of the curves it is seen that the initial rates are the more critical for comparison of differences in activity. Thus, the initial rate for the diluted (1:10) filtrate of *Aspergillus fumigatus* is just one-tenth that of the undiluted filtrate. This proportionality, however, does not persist on longer incubation (figure 2). The results of one test (figure 2) involving filtrates of

different origins show the same relative activity for four of the filtrates, whenever they are compared. The fifth, a filtrate obtained by growing a non-cellulose-destroying fungus (*Aspergillus sydowi*) on CMC, diminishes from second in order of activity at 15 minutes, to third in 2 hours, and to fourth in 3 hours.

Effect of pH. The effect of pH on Cx activity has been determined (figure 3). It is clear from the graph that various filtrates show different responses to pH changes. The filtrates from all four organisms are satisfactorily assayed at pH 5.1. It is quite likely that filtrates of certain bacterial cultures may require more alkaline conditions. Phosphate buffers (M/10) have been used throughout this phase of the work, but citrate buffer has subsequently been found superior in tests where phosphate may precipitate.

The pH-Cx activity curve of *Aspergillus fumigatus* filtrates follows closely that previously determined for cellulolytic activity on filter paper (Reese,

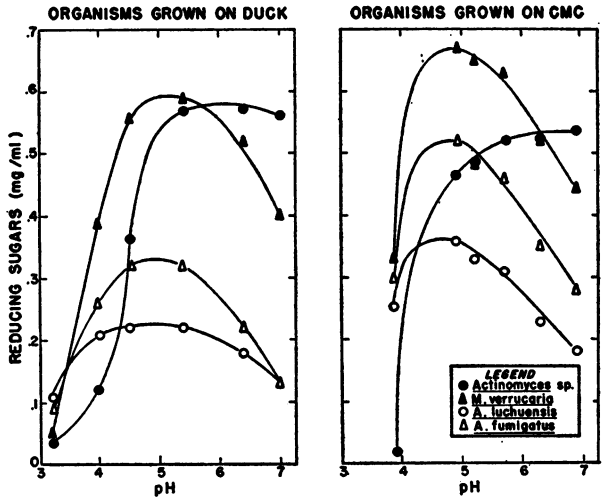


Figure 3. Effect of pH on Cx activity of various filtrates. Time, 1 hour; temper ature, 50 C.

1946). The Cx activity curve for *Myrothecium verrucaria* resembles that reported by Saunders *et al.* (1948), using ball-milled cotton, but has a slightly lower optimum pH. The relative inactivity of the fungus filtrates at pH 7.0 to 8.0 coincides with the known preference of these organisms for acid conditions, and the high activity of the *Actinomyces* sp. filtrate at pH 7.0 to 8.0 with the preference of that organism for neutral to slightly alkaline conditions. Though the correlation is good, this in no way explains why these filtrates behave differently.

In general, the pH stability curves for Cx are much broader than the pH activity curves obtained at the same temperature (figures 3, 4). At lower hydrogen ion concentrations, however, the types are similar in that both stability and activity drop off rapidly below pH 4.5. The lower Cx activity at pH 4.0 cannot be explained as a result of inactivation of enzyme, since activity is de-

termined in a short (2-hour) assay, whereas an incubation period of 22 hours at pH 4.0 is necessary to demonstrate a measurable effect on enzyme inactivation. The hydrolytic enzyme, Cx, of *Aspergillus luchuensis* is stable at pH 7.0 (but much less so at pH 8.0), yet the activity at this pH is quite low. It appears, therefore, that inactivation in no way accounts for the shape, or limits, of the pH activity curves for the enzyme Cx.

The same relationship of stability and activity curves was previously noted for *Aspergillus fumigatus* filtrates (Reese, 1946) when cellulolytic degradation of filter paper (instead of CMC) was measured. Both curves taper off below pH 4.7, but, although the activity curve fell off rapidly as the pH rose from 5.0 to 7.0, the stability curve did not.

All of the stability tests discussed above were performed in the absence of substratum. The interrelationships between the various factors are partially

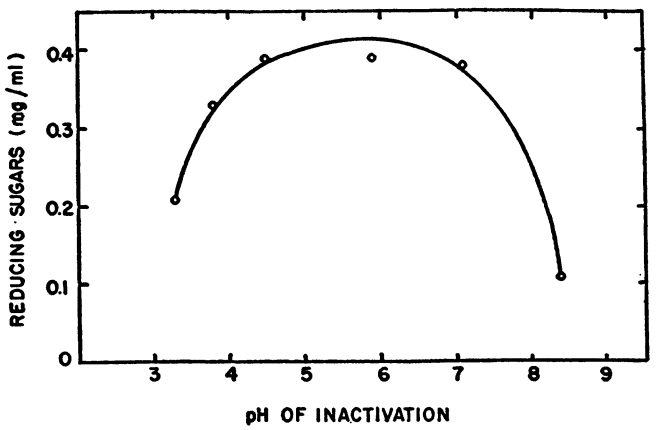


Figure 4. Effect of pH on Cx stability. *Aspergillus luchuensis* filtrates inactivated at 50 C for 22 hours; readjusted to pH 5.1; and tested against CMC 50 T at 50 C, 1 hour; final phosphate concentration is the same at all pH levels.

explained by some results of experiments showing the action of filtrates on ground dewaxed cotton sliver versus time, temperature, and pH. A measure of stability is obtained by comparing the amount of sugar formed during the first 3 days with that formed during the next 3 days (table 4). At pH 5.2 it is apparent that the inactivation rate is much greater at 50 than at 40 C.

The filtrates vary in the rate at which each is inactivated. The most resistant filtrates are those of *A. fumigatus* and *A. luchuensis*; those of *M. verrucaria* and *Actinomyces* sp. are most readily inactivated. These data, showing inactivation in the presence of substratum (cotton), are injected here for comparison with data obtained from CMC (Cx activity), with which they are in agreement. Both CMC and cotton sliver are hydrolyzed more rapidly at pH 5.2 than at pH 7.5.

Effect of temperature. Several filtrates obtained by growing organisms on cotton duck have been tested for Cx activity at various temperatures (figure 5).

Since the stability and activity of Cx are greatest near pH 5.1, the CMC solutions were adjusted to this pH with phosphate buffer (M/20). The data plotted were obtained at the end of 1 hour. Three filtrates were most active at 60 C, which represents the highest temperature in the test, and the fourth at 52 C. Other studies have shown maximum hydrolysis rates at 65 C, when a 30-minute in-

TABLE 4

Effect of pH and temperature on cellulolytic action of filtrates (on cotton)

FILTRATE FROM	REDUCING SUGAR, MG/ML							
	pH 5.2				pH 7.5			
	40 C		50 C		40 C		50 C	
	Days		Days		Days		Days	
	0—3	3—6	0—3	3—6	0—3	3—6	0—3	3—6
Actinomyces sp.	0.28	0.13	0.40	0.00	0.17	0.10	0.27	0.06
Aspergillus fumigatus	0.35	0.32	0.65	0.30	0.14	0.05	0.20	0.07
Myrothecium verrucaria	0.28	0.11	0.27	0.03	0.16	0.06	0.07	0.00
Aspergillus luchuensis	0.13	0.09	0.22	0.10	0.03	0.03	0.00	0.00

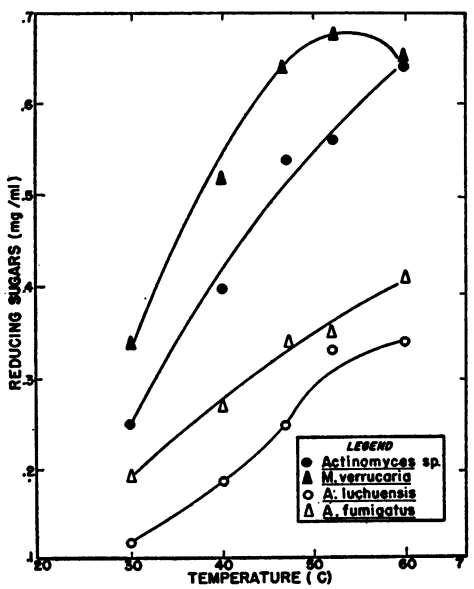


Figure 5. Effect of temperature on Cx activity of various filtrates. Incubation for 1 hour at pH 5.1 with CMC 50 T.

cubation time was used. With longer periods of incubation there is some inactivation of the filtrates giving an apparent fall in the optimum temperature for hydrolysis.

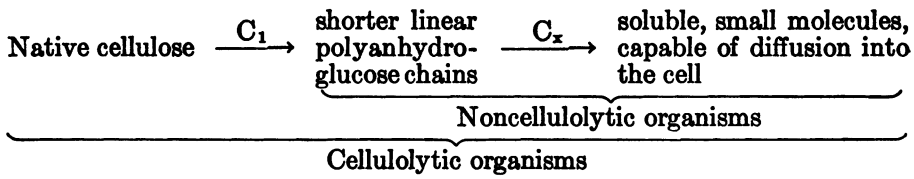
These data are in general agreement with those for the activity of the same filtrates on ground cotton (table 4) kept at pH 5.1 for 3 days. The rate of hydrol-

ysis is much greater at 50 than at 40 C for filtrates of three organisms. The filtrate of the fourth, *M. verrucaria,* shows an optimum between 40 and 50 C. This is somewhat below the value for Cx activity obtained in 2 hours but above that given by Saunders *et al.* (1948) for this organism. It appears that inactivation proceeds much more rapidly in filtrates of *M. verrucaria* than in those of the other organisms studied. Since frequent comparisons are being made with the work of Saunders with this same organism, it should be pointed out that his preparations were dialyzed whereas ours were not. We have repeated our tests, however, and find that dialyzed filtrates behave in essentially the same manner as those that have not been dialyzed.

Effect of other factors. As a preservative in all of our solutions, merthiolate is used at a concentration of 0.01 per cent. This germicide protects both the stock CMC solution and the cell-free filtrates at refrigerator temperature for long periods of time. No diminution in the Cx activity of filtrates so preserved has been observed within a 6-month period.

DISCUSSION

The data presented indicate that the ability to develop an enzyme capable of hydrolyzing the 1,4-β-glucosidic linkage found in cellulose and its derivatives is widespread among microorganisms. The ability of microorganisms to use native cellulose as a substratum is more restricted. Thus, noncellulolytic fungi may utilize CMC. From these facts it is postulated that the classical enzyme "cellulase" (Pringsheim, 1912), presumably converting native cellulose to sugars, consists of at least two systems. The first step, designated as C_1, occurs preliminary to hydrolysis of the straight chain by Cx. The sequence is diagramed as follows:



This hypothesis readily explains the observations of Seillière (1906, 1907). He found that native cellulose passes unchanged through the digestive tract of the snail. However, cellulose preparations obtained by reprecipitation from solutions of Schweitzer's reagent are easily digested by the snail as well as by crude snail enzymes. Riere (1939) also implied that certain species of *Mucor* and *Aspergillus* are capable of attacking "hydrocelluloses" but not "highly polymerized" cellulose. Our own results are in harmony with these observations.

Throughout this work the assumption is made that Cx, the enzyme hydrolyzing the 1,4-β-glucosidic linkage in CMC, is also the enzyme attacking the same linkage in cellulose. This belief is based on the presence of Cx in the filtrates of cellulolytic organisms grown on cellulose. In other tests (unreported) it was found that Cx is not produced by the same organism grown in the *absence*

of substances containing the particular linkage that is attacked. Thus, no Cx was obtained when glycerol was the substratum, though good growth took place. In other words, Cx is produced apparently in response to the presence of the β-glucosidic linkage, whether that linkage is in cellulose, in hydroxyethyl cellulose, or in carboxymethyl cellulose, and it is *not* specific for the modified chain.

The products of hydrolysis of CMC by the cell-free filtrates are unknown. The filtrates produce reducing compounds from CMC and from cellulose. Glucose appears to be absent from the hydrolytic products of CMC but present in the hydrolyzates of cellulose (by glucosazone tests, unreported). None of the filtrates reported herein have cellobiase activity as measured by an increase in reducing sugars. It is believed that Cx has the ability to produce glucose as an end product from the long-chain compounds but that it is unable to hydrolyze cellobiose. It appears that cellobiase plays little, if any, part in the hydrolysis.

The hydrolytic enzyme, Cx, is unable to hydrolyze starch, pectic acid, alginic acid, or bacterial dextran.[4] It is produced by all cellulolytic organisms tested and by some noncellulolytic ones. Its pH activity, pH stability, and temperature-activity ranges appear to coincide with those of "cellulase."

The precise action of C_1 is not clear at the present time. It may be concerned with a splitting of the cross linkages postulated in native cellulose by Pacsu (1947), Hess and Steurer (1940), and Haworth (1939), and of other nonglycosidic linkages (Schulz and Husemann, 1942). In any case, the resultant is a degradation of the native cellulose molecule into linear polysaccharide anhydroglucose chains.

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SUMMARY

The degree of substitution of soluble cellulose derivatives is a primary factor in determining the resistance of these materials to microbial attack. The presence of a single substituent on every anhydroglucose unit appears to confer immunity. Below this threshold value, increasing the DS of the cellulose derivative increases its resistance. Methyl cellulose, with the high DS required for solubility, is usually resistant to microbial attack. Carboxymethyl cellulose and hydroxyethyl cellulose, as they appear on the market, are readily hydrolyzed by microorganisms.

The ability to hydrolyze the 1,4-β-glucosidic linkage found in cellulose is *not* limited to cellulose-decomposing organisms. Noncellulolytic organisms grown on CMC (or on hydroxyethyl cellulose) also attack that linkage and produce filtrates having Cx activity.

[4] Dextran A, from *Leuconostoc mesenteroides*, furnished by Dr. Allene Jeanes, 1949.

Biological solubilization of cellulose probably takes place in at least two steps: (a) conversion of the native cellulose molecule into linear anhydroglucose chains and (b) hydrolysis of the 1,4-β-glucosidic linkage to form soluble sugars.

A rapid and convenient procedure for the estimation of the enzyme Cx hydrolyzing the 1,4-β-glucosidic linkage is described. The procedure makes use of soluble cellulose derivatives as substrata.

REFERENCES

FREEMAN, G. G., BAILLIE, A. J., AND MACINNES, C. A. 1948 Bacterial degradation of CMC and methyl ethyl cellulose. Chemistry & Industry, 1948, 279–282.

GREATHOUSE, G. 1949 Microbiological degradation of cellulose. Report at Am. Chem. Soc. Meeting, Atlantic City, September, 1949.

HARRY, JOHN 1948 *Personal communication.*

HAWORTH, W. N. 1939 The structure of cellulose and other polymers related to simple sugars. Chemistry & Industry, **17**, 917–925.

HESS, K., AND STEURER, E. 1940 Vergleich von Endgruppenbestimmung und Viscosität bei Cellulose. Ber. deut. chem. Ges., **73B**, 669–676.

MORGAN, P. W. 1946 Determination of ethers and esters of ethylene glycol. Ind. Eng. Chem., Anal. Ed., **18**, 500–504.

PACSU, E. 1947 Molecular structure of cellulose and starch. J. Polymer Sci., **2**, 565–582.

PIGMAN, W. W., AND GOEPP, R. M. 1948 Chemistry of the carbohydrates. Academic Press, Inc., New York.

PRINGSHEIM, H. 1912 Über der fermentativen Abbau der Zellulose. Z. physiol. Chem., **78**, 266–291.

REESE, E. T. 1946 Decomposition of cellulose by microorganisms at temperatures above 40°C. Thesis, Pennsylvania State College.

RIERE, J. 1939 Les moisissures: conditions de développement, identification des défauts, remèdes. Rev. gén. mat. color., **43**, 441–444.

SAUNDERS, P., SIU, R. G. H., AND GENEST, R. N. 1948 A cellulolytic enzyme preparation from *Myrothecium verrucaria.* J. Biol. Chem., **174**, 697–703.

SCHULZ, G. V., AND HUSEMANN, E. 1942 Molecular weight distribution in decomposed cellulose. Z. physik. Chem., **B52**, 23–49.

SEILLIÈRE, G. 1906 Sur un cas d'hydrolyse diastasique de la cellulose du coton, après dissolution dans la liqueur de Schweitzer. Compt. rend. soc. biol., **61**, 205–206.

SEILLIÈRE, G. 1907 Remarques sur l'hydrolyse diastasique de la cellulose du coton et quelques autres polysaccharides. Compt. rend. soc. biol., **63**, 515–517.

SIU, R. G. H., DARBY, R. T., BURKHOLDER, P. R., AND BARGHOORN, E. S. 1949 Specificity of microbiological attack on cellulose derivatives. Textile Research, **19**, 484–488.

SPURLIN, H. M. 1939 Arrangement of substituents in cellulose derivatives. J. Am. Chem. Soc., **61**, 2222–2227.

SPURLIN, H. M. 1949 *Personal communication.*

SUMNER, J. B., AND SOMERS, G. F. 1944 Laboratory experiments in biological chemistry. Academic Press, Inc., New York.

WOODWARD, ROLAND 1948 *Personal communication.*

ZIESE, W. 1931 Über die Einwirkung von Fermenten des Magensaftes von *Helix pomatia* und solcher des Gerstenmaldes auf Celluloseglykoläther. Z. physiol. Chem., **203**, 87–116.